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**IMAGING SYSTEM AND METHOD FOR BIOMEDICAL ANALYSIS**

**STATEMENT OF GOVERNMENT INTEREST**

**[0001]** The invention described herein may be manufactured and used by or for the Government of the United States of America for governmental purposes without the payment of any royalties thereon or therefor.

**CROSS REFERENCE TO OTHER PATENT APPLICATIONS**

**[0002]** None.

**BACKGROUND OF THE INVENTION**

(1) Field of the Invention

**[0003]** The present invention relates to a system and method of shadow imaging for biomedical analysis, and more particularly, to an optical cytology device for analyzing biomedical samples.

(2) Description of the Prior Art

**[0004]** Conventional methods of screening biomedical samples for pathogen-borne diseases generally involve a technician manually counting the cells under a microscope. Such manual counting methods utilizing the best medical standards result in an accuracy rating of approximately 87% and the process usually takes > 1 hour to complete.

**[0005]** Certain prior art systems have attempted to improve the screening of biomedical samples. For example, U.S. Patent 7,911,617, issued to Padmanabhan et al. provides a system for scattered light and simultaneous multi-color fluorescent light detecting for analyzing, classifying, and identifying biological particles and items of interest. Broadly, Padmanabhan et al. utilize the diffraction of a laser light source in flow cytometry to count cells or particles in a flowing biological sample. However, flow cytometry cannot give the same accuracy as a manual cell count.

**[0006]** Another prior art concept is a lensless imaging system proposed in a research paper entitled "Lensless wide-field fluorescent imaging on a chip using compressive decoding of sparse objects" by A. Coskum et al. 136 Analyst No. 17, pp. 3512-3518, (7 September 2011). Their fluorescent microscopy lensless imaging approach utilizes a large-format CCD sensor and can be applied to blood samples in micro-fluidic chips. In this technique, a fluorescent pump light is applied to the sample to cause cells to fluoresce. The pump light isn't transmitted through to the CCD sensor. An on-chip fiber optic faceplate is used to collect light from sample over an ultra-large field of view of (up to  $> 8 \text{ cm}^2$ ), rather than collecting the light directly at the CCD. Furthermore, this technique utilizes a compressive sampling algorithm to rapidly reconstruct the sparse distribution of

fluorescent sources. This algorithm is not strictly designed for cell counting.

**[0007]** U.S. Patent Publication 2011/0183370, to Noiseux et al. provides an optical imaging system for identifying bacterial cells in food samples, the bacterial cells being labeled with fluorescent nanoparticles. Generally, Noiseux et al. teach injecting multiple fluorescent nanoparticle dyes into the food sample, imaging the sample a number of times using multiple light sources having different wavelengths, and then indentify the target cells through differentiation of the resultant images. However, the system disclosed by Noiseux is complicated, costly, and generally does not disclose a shadow imaging system for biomedical analysis.

**[0008]** An article published by Sang Jun Moon et al., "Integrating Micro-fluidics and Lensless Imaging for Point-of-Care," 24 *Biosens Bioelectron* No. 11, pp. 3208-3214 (15 July 2009), teaches an integrated platform that merges a micro-fluidic chip with lensless imaging for cell counts. The cells are detected through shadow imaging of an optically clear chip. Shadows of the cells are reproduced as grayscale images on a CCD sensor. Software was used to automate the cell counting process.

**[0009]** Thus, there is a need to for an improved system and method to screen biomedical samples for pathogen-borne diseases, such as is identified in the present disclosure.

### **SUMMARY OF THE INVENTION**

**[0010]** Accordingly, it is an object of the present invention to provide a device and method for the detection and diagnosis of diseases such as, for example, AIDS, malaria, cholera, lymphoma, and typhoid. The present disclosure can be used to capture and count microscopic cells for application as a biomedical device for screening samples for pathogens, infected cells, abnormal lymphocytes and other purposes. Using the presently disclosed system and method, accurate cell counts can be obtained compared to manual methods. This system was found to be highly accurate, with <1% error compared to 17% for an earlier implementation and ~13% for counting under a microscope. In addition, the automated cell counting process takes only seconds, compared more than an hour for manual counting.

**[0011]** Another object of the present disclosure is to provide a method of determining the quantity of a selected cell type in a sample. The method may comprise providing a sample into an analysis chamber having a stain or reagent material. The sample can include a plurality of sub-chambers such that the stain or reagent material can be selected so as to label a target cell type (e.g., to render at least one of the cell types in the sample more visible with respect to the other cell types in the sample). The method may further comprise capturing an image of the sample based on a uniform planar light being projected through the analysis

chamber containing the sample and onto the image sensor. The image can be captured by an image sensor. The method may also comprise analyzing the image, via an image processor, so as to identify the target cell type (e.g., the target cell type rendered more visible than the other cell types by the stain or reagent). The method may even further comprise providing information indicative of the quantity of the target cell types, i.e., how many of the target cell types are contained in the sample.

**[0012]** Yet another object of the present disclosure is to provide an optical cytology system. The system may comprise a micro-fluidic chip. The micro-fluidic chip may comprise an analysis chamber for receiving a sample having a plurality of cell types. The chip may further comprise a stain or reagent provided in the analysis chamber and adapted to label a target cell type (e.g., to render the target cell type more visible with respect to other cell types in the sample). The system may further comprise a large format light source [e.g., an array of light-emitting diodes (LEDs)] configured to project light through the analysis chamber containing the sample. The system may even further comprise an image sensor configured to generate an image based on the light being projected through the analysis chamber containing the sample.

**[0013]** The system may also comprise an image processor configured to analyze the image to determine the quantity of

target cell types in the sample. The entire system is relatively inexpensive, compact, and designed to be portable, which is beneficial for ease of transportation to testing site. It is also entirely self-contained, consumes low power, and may be battery operated.

**[0014]** A further object of the present disclosure is to provide an optical cytology system. The system may comprise a micro-fluidic chip and an image processor. The micro-fluidic chip may comprise at least two analysis chambers for receiving a sample in each analysis chamber. The sample can have a plurality of cell types, such as CD4<sup>+</sup> cells or lymphocytes in whole blood. The chip may also comprise a stain or reagent provided in each of the analysis chambers and being adapted to label a target cell type (e.g., to render the target cell type more visible with respect to other cell types within the analysis chamber). Each analysis chamber can include a different stain or reagent so as to label different target cell types within each chamber. The system may further comprise a light source configured to project a light through each of the analysis chambers. The light source may be a large format light source such as an array of light-emitting diodes (LEDs). The system may even further comprise an image sensor configured to generate an image based on the light being projected through each of the analysis chambers containing the sample. The image processor can be configured to analyze the image



to determine the quantity of target cell types in each of the analysis chambers dependent upon the stain or reagent in said chamber.

**[0015]** An even further object of the present disclosure is to provide capture techniques for sorting and isolating desired cells from non-specific cells or bacteria present so as to optimize the accuracy of cell count of the desired cell type. These capture techniques may include, but are not limited to, antibody-specific reactions using surface chemistry or surface coating, using size-exclusion channels, and sheath flow and streamline sorting.

**[0016]** Other objects, features, and advantages of the present invention will be apparent to those having ordinary skill in the art reading the instant specification, drawings, and appended claims.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

**[0017]** FIG. 1 is a is a diagram of an exemplary optical cytology system constructed in accordance with the present disclosure;

**[0018]** FIG. 2 shows exemplary steps illustrating formation of a micro-fluidic device in accordance with the present disclosure;

**[0019]** FIG. 3 is a perspective view of a micro-fluidic chip and imager in accordance with the present disclosure;

**[0020]** FIG. 4 is an alternate embodiment of a micro-fluidic device in accordance with the present disclosure;

**[0021]** FIG. 5 is an exemplary image generated in accordance with the present disclosure;

**[0022]** FIG. 6A and 6B are two images showing the original image of FIG. 5 at 6A and then a processed bitmap image at 6B; and

**[0023]** FIG. 7 is an exemplary flowchart of a method of determining the quantity of a target cell type in a sample, in accordance with aspects of the present disclosure.

#### **DETAILED DESCRIPTION OF THE INVENTION**

**[0024]** Referring now to the drawings, and more particularly to FIG. 1, shown therein is a block diagram of an exemplary optical cytology system 100 constructed in accordance with the present disclosure. The system 100 may comprise a micro-fluidic chip 102, an imager 104, and a processor 106. An identifying tag 102A can be positioned on micro-fluidic chip 102. Imager 104 can have a tag reader (not shown) incorporated within. As an alternative, tag 102A can be an optical tag that is imaged when chip 102 is imaged. Tag 102A and tag reader can be any optical, magnetic, chemical, radio or mechanical system known in the art. The system 100 can be used to capture and count microscopic cells for application as a biomedical device for screening samples for pathogens and other

purposes. The system 100 can be utilized in the area of optical material analysis and biomaterial cell identifying, counting, and evaluating.

**[0025]** Broadly, a liquid specimen is provided to a micro-fluidic chip which can be automatically optically analyzed. The liquid specimen can be a biological fluid such as whole blood. These cells may be imaged using a shadow imaging system 104 having a lensless charge coupled device (CCD) sensor or other light sensor. With this shadow imaging technique, automated cell counts are performed after whole blood has been injected into the micro-fluidic chip 102. The imager 104 obtains a digital image of the specimen in micro-fluidic chip 102. This image can be provided to a local or remote processor 106 for analysis. Processor 106 has an algorithm that can isolate visual anomalies from their surroundings based on, for example, the different levels of contrast. The imager 104 can include a chip platform allowing the insertion of micro-fluidic chip 102. The imager 104 can include a light source positioned on one side of the chip 102 and an image sensor positioned on the other side of the chip 102. After chip 102 is positioned in the imager 104 an image is obtained from the sensor. The image captured by the sensor can include the diffracted shadow signals of cell shape that are present in the specimen. The biomedical cell analysis computer algorithms on processor 106 are capable of effectively identifying cells from

nonuniform backgrounds based on either the Gaussian nature of their intensity or the contrast of their amplitude with that of surrounding pixels.

**[0026]** FIG. 2 is a cross-sectional view illustrating a micro-fluidic device 200, in accordance with the present disclosure. As will be discussed later, the micro-fluidic device 200 forms at least a portion of the micro-fluidic chip 102 of the present disclosure. Device 200 includes a cover 204. An inlet 202 and an outlet 206 are formed in cover 204. Cover 204 can be made from a plastic, glass or some other transparent material. Preferably, cover 204 is made from a polymethyl methacrylate (PMMA) material, e.g., a biocompatible, acrylic glass. Cover 204 can be formed from a material or cut from a sheet of material. Preferably, inlet 202 and outlet 206 have a diameter of about 0.8 mm. In one embodiment, cover 204 has a thickness 208 of about 3.175 mm. At least a portion of the cover 204 must be transparent. In one embodiment, all of the cover 204 is transparent.

**[0027]** An adhesive 210 having a thickness 212 is used to join cover 204 to a base plate 214. Adhesive 210 is preferably a double-sided adhesive tape having a thickness 212 of, for example, about 50  $\mu\text{m}$ . Using tape as an adhesive allows a more consistent separation between base plate 214 and cover 204. Base plate 214 is sealed against cover 204 by the adhesive 210. Base plate 214 can have a thickness 216 of, for example, about 100  $\mu\text{m}$ . At least a

portion of the base plate 214 must be transparent. In one embodiment, the entire base plate 214 is transparent. Base plate 214 should be formed from glass or a rigid, transparent plastic.

**[0028]** The region between cover 204 and plate 214 and surrounded by adhesive 210 defines an analysis chamber 218. The inlet 202, the analysis chamber 218 and the outlet 206 are in fluid communication. That is, a fluidic sample can enter the inlet 202, flow into the analysis chamber 218 for analysis, and then be discharged via the outlet 206. Outlet 206 can also provide an egress for gas or fluid present in the analysis chamber 218 before introduction of a specimen. Additionally, a positive pressure can be provided to push specimen into inlet 202 or a suction or negative pressure can be provided at outlet 206 to aid in communication of a specimen into analysis chamber 218. An inlet tube 224 can be mounted to the inlet 202 and, similarly, an outlet tube 226 can be mounted to the outlet 206. The inlet and outlet tubes 224 and 226, respectively, can be mounted to the cover using, for example, a liquid adhesive 228 such as epoxy.

**[0029]** Referring now to FIG. 3, shown therein is a perspective view of an exemplary micro-fluidic chip 102 in accordance with the present disclosure. Generally, the system 100 consists of an imaging system and a micro-fluidic device 102. The imaging system comprises a light source 308 and an image sensor 306. The micro-fluidic device 102 can be the micro-fluidic device 200 illustrated

in FIG. 2. That is, the micro-fluidic device 102 can comprise an inlet 202, an outlet 206, and an analysis chamber 218 that are in fluid communication so as to receive a sample for analysis.

Generally, the micro-fluidic device 102 can be positioned between the light source 308 and the image sensor 306. As such, the light from the light source 308 is projected through the micro-fluidic device 102 (e.g., through the analysis chamber 218 containing the sample) and onto the image sensor 306.

**[0030]** The light source 308 can be implemented using a large format LED array. This type of light source projects a uniform spread of light from multiple LED devices over the entire sample surface. Preferably, light source 308 projects a full spectrum white light. Light source 308 should have at least as large a planar area as that of the portion of the analysis chamber 218 being analyzed. The shadow imaging characteristics and associated cell contrast are typically better than those designs using a single point source. The use of a point light source causes problems with parallax and diffraction that can result in an image that is more difficult to analyze. Because of parallax, a point light source must be located sufficiently far from the cell to act effectively as a planar light source. A uniform, planar light source can be located closer to the slide. The effects of diffraction are eliminated by the diffuse light source. This results in a Gaussian intensity profile on the sensor. The use of

polarized light may also be incorporated to likewise further enhance the contrast. The light being projected from the light source 308 is transmitted through the analysis chamber 218 of the micro-fluidic device 102.

**[0031]** The image sensor 306 can be positioned on an opposing side of the micro-fluidic device 302 such that the light being projected through the analysis chamber 218 containing the sample casts a shadow 310 of a target cell type 312 onto the image sensor 306. The image sensor 306 can be implemented using a CCD or CMOS. The image sensor 306 can be a lensless image sensor. The image sensor 306 is configured or otherwise adapted to generate an image. In one embodiment, the image sensor 306 is configured to generate a high resolution digital image, e.g., an image having a resolution of 10,000 by 10,000 pixels. The image sensor 306 should have an area at least as large as that of the region of analysis chamber 218 being analyzed. The image may be generated based on the light being projected through the analysis chamber 218 containing the sample. The analysis chamber 218 of the micro-fluidic device 102 may include a stain or reagent adapted to label a target cell type in the sample. That is, the stain or reagent may be configured to render the target cell type more visible with respect to the other cell types in the sample. As such, the target cell types rendered more visible cast a shadow onto the image sensor 306. The image generated by the image sensor 306 will

include information indicative of the shadows being cast by the target cell types.

**[0032]** In operation, a sample such as a hematology sample is placed into the micro-fluidic device 102 through input 202. The sample may be obtained directly from a patient. As illustrated in FIG. 3 and understood in the art, the hematology sample may include, for example, red blood cells, white blood cells (which may include lymphocytes which are relatively large and easily detectable), T-helper cells, etc., as well as the target cell type. The sample after being provided at inlet 202 of the device 102 flows to the analysis chamber 218. The analysis chamber 218 can contain a stain or reagent material. As would be understood, utilization of a stain is a technique used to highlight the structure, or otherwise enhance the contrast of a target cell type. A reagent material is generally understood to refer to a substance or compound added to a sample so as to bring about a reaction with a target cell type. In the instant application, said stain or reagent may be selected based on the target cell type being tested for. In one embodiment, the target cell type is a bacteria cell.

**[0033]** Once the sample has been introduced into the analysis chamber 218 of the micro-fluidic device 102, the device 102 is positioned between the light source 304 and the image sensor 306. The light source 304 is activated so as to project light through



the analysis chamber 218 containing the sample. The light continues from the analysis chamber 218 and onto the image sensor 306. The target cell types dyed by the stain or reagent create shadows on the image sensor 306, which are captured in the image generated by the image sensor 306. That is, the image created by the image sensor 306 includes information indicative of the diffracted shadow signal 310 of the target cell type 312.

**[0034]** Referring now to FIG. 4, shown therein is an alternate embodiment of a micro-fluidic device 400 in accordance with the present disclosure. In this embodiment, the device 400 is configured to test for more than one target cell type. In the exemplary embodiment illustrated in FIG. 4, the device 400 comprises three analysis chambers 402A, 402B and 402C that are in fluid communication with a specimen port 404 via one or more inlet tubes 406. Although the alternate embodiment illustrated in FIG. 4 shows use of three analysis chambers 402A, 402B and 402C, it is to be understood that any number of analysis chambers 402A, 402B and 402C may be included in the device 400. The use of multiple analysis chambers allows multiple types of target cells to be analyzed and quantified using a single sample. In a preferred embodiment, each of the analysis chambers 402A, 402B and 402C may include a different stain or reagent such that different target cells are labeled. For example, a first analysis chamber 402A may include a stain or reagent adapted to label a first cell type, a

second analysis chamber 402B may include a stain or reagent adapted to label a second cell type, and so on. As such, the embodiment illustrated in FIG. 4 can be used to allow for multiple analyses to be conducted in parallel.

**[0035]** The specimen port 404 is adapted or otherwise configured to receive a sample (e.g., a hematology sample) for analysis. At least a portion of the sample will flow through some of the inlet tubes 406 and into each of the analysis chambers 402A, 402B and 402C so as to be exposed to the stain or reagent. Once testing is complete, the sample can flow through at least another portion of the outlet tubes 408 for discharge.

**[0036]** The alternate embodiment illustrated in FIG. 4 also includes an optional feature of the present disclosure wherein the micro-fluidic device 400 includes a flushing system. It is to be understood that any of the herein described micro-fluidic devices may include such a flushing system. Generally, the flushing system comprises a storage reservoir 410 storing a flushing liquid and a disposal reservoir 412. The storage reservoir 410 storing the flushing liquid is configured or otherwise adapted to permit the user to release the flushing liquid into the fluid channels 406 so as to flow into the analysis chambers 402A, 402B and 402C and, subsequently, into the disposal reservoir 412. As would be understood, the flushing fluid may be adapted to sterilize or otherwise render safe the analysis chambers 402A, 402B and 402C as

well as the sample contained therein. In one embodiment, the flushing liquid is a saline solution.

**[0037]** When utilizing the alternate embodiment of the device 400, the light source 304 and image sensor 306 of the present disclosure would be configured to operate with the multiple analysis chambers 402A, 402B and 402C. For example, the light source 304 may be configured to project the light source through each of the analysis chambers 402A, 402B and 402C and onto the image sensor 306. Other aspects may provide for multiple light sources 304 being used such that each analysis chamber 402A, 402B and 402C has its own light source 304 projecting light thereon. Similarly, the image sensor 306 may be configured so as to operate with the multiple analysis chambers 402A, 402B and 402C. In one aspect, the image processor (discussed below) may be configured so as to differentiate different regions of the image according to which analysis chamber 402A, 402B and 402C the image sensor 306 is positioned under. Other aspects may provide for separate image sensors 306 to be used such that each analysis chamber 402A, 402B and 402C is imaged.

**[0038]** Referring now to FIG. 5, shown therein is a representation of an image 500 generated in accordance with the present disclosure. Representative image 500 is a portion of analysis chamber 402A identified as 5 in FIG 4. The image 500 can be generated or otherwise created by the image sensor 306. In the

representative image shown in FIG. 5, the image 500 includes information indicative of the target cells 502 being labeled in one of the analysis chambers 402A, 402B and 402C. The representation shows the target cells 502 subjected to a biological stain or reagent to increase their visibility. The stained target cells 502 may create a shadow on the image sensor 306 that is displayed in the resulting image.

**[0039]** The image 500 created by the image sensor 306 is provided to the image processor 106 (illustrated in FIG. 1). Generally, the image processor 104 can be connected to, or otherwise in electrical communication with the image sensor 306 so as to receive the image 500 therefrom. Other aspects may provide for the image sensor 306 to be configured to store the image 500 in a digital format on a storage device (e.g., SD card, CD-R/RW disc, magnetic disk drive, etc.). In this embodiment, the storage may then be provided to the image processor 106 for analysis of the image 500.

**[0040]** The image processor 106 may be implemented as a software module being executed on a computerized processing system (not shown). The image processor 106 may be implemented via a series of computer executable code being executed by a processor of a computer so as to implement portions of the present disclosure. The computerized processing system may be a general purpose computer, laptop, netbook, smart phone, tablet computer, etc.

Other aspects may provide for the image processor to be implemented as executable code operating on a real and/or virtual server. The server can be in communication via a network, cellular communications network, etc. The computerized processing system may include a storage device, a user input/output device, a display, etc., such that a user can interact with/control the computerized processing system to implement aspects of the present disclosure. In a preferred embodiment, the computerized processing system will comprise a mechanism to output information (e.g., visual display, hard print, etc.) to the user that is indicative of the quantity of target cell types that are contained in the sample.

**[0041]** The image processor 106 is adapted to process the image 500 to identify target cells 502 from non-uniform backgrounds based on either the Gaussian nature of their intensity or the contrast of their amplitude with that of surrounding pixels. In the one case, the image processor 106, starting at each and every pixel, looks for a Gaussian profile, of increasing and then decreasing contrast, that would indicate the captured image of a cell object. The processor can likewise detect the incrementally more complex Gaussian profiles for ring shaped cell objects. In the another instance, the image processor, for each pixel in the captured image, searches for surrounding pixels in all four directions a specified separation away, and compares the contrast

with that of the original pixel. If all four (assuming a internally located pixel) contrast differentials are greater than a specified value, that pixel is considered to be at or near the center of a target cell object. It is expected that in one embodiment, the specific analysis routine can be chosen based on the target cell size and the pixel size. Intensity or contrast based thresholding can be used for cells that are between one and three pixels in size. Gaussian profile analysis can be performed when the target cell features are larger than three pixels in a single dimension.

**[0042]** In order to find a ring shaped cell object, the image processor can look for two more narrow Gaussian profiles separated by a predetermined number of pixels in both (x and y) directions. The inclusion of ring shaped cell patterns, as well as the pixel separation between the two Gaussian peaks representing points on the cell perimeter, may be specified as an input parameters to the image processor to optimize the accuracy of the cell counting algorithm. The pixel separation can be determined based on the targeted cell and experimental results.

**[0043]** The image processor 106 can thus be adapted to isolate a pixel, or group of pixels belonging to a target cell and then group adjoining pixels as comprising a target cell object. Based on the number of target cell objects, the image processor 106 can determine the quantity of the target cells contained in the

sample. Other parameters can also be determined such as target cell density.

**[0044]** The image processor 106 can process the image 500 utilizing steps of converting the image 500 to grayscale format in matrix form. The processor can then scan through the captured image matrix. For each current pixel, the image processor 106 compares the contrast level of that pixel with that of pixels an arbitrary pixel separation  $\Delta p$  above, below, to the left, and to the right.  $\Delta p$  is dependent on average cell size and sensor density. In one example,  $\Delta p$  = three pixels. If the contrast difference for each of the four calculations is greater than a specified threshold level, that pixel is considered as comprising a feature, e.g., a target cell. Boundary conditions can also be utilized for pixels where one or more pixels a distance  $\Delta p$  away in the four directions are beyond the image dimensions.

**[0045]** Utilizing color and/or grayscale images allows distinction of cells when the change between a cell and the background is subtle at the boundaries. This cell detection is based on the relative or local contrast between cells and the background. This allows analysis of an image featuring a spatially varying background which cannot be converted into a binary image accurately. The image processor is also capable of determining the physical dimensions of cell objects given the proper conversion input data, which allows the operator to specify

the expected cell dimensions (e.g., in microns) rather than number of pixels.

**[0046]** A representation of this processing on the image of FIG. 5 can be shown in the comparison of the representations shown in FIG. 6A and FIG. 6B. FIG. 6A shows the representation of FIG. 5, image 500 before processing. FIG. 6B shows the representation of FIG. 5 after processing, giving processed image 600. In an actual test, an original image was made of labeled neutrophils and the quantity of target cells counted using the image processor of the present disclosure, with  $\Delta p = 3$  pixels, was 275 cells. This result was confirmed by manually counting the target cells labeled by the stain or reagent. This processing has also been tested on an image having a background that varies substantially across the image. It was found that, while the background illumination of the image varies greatly, the majority of stained target cells can be detected and counted using aspects of the present disclosure.

**[0047]** Image processor 106 can use other variations for image processing. In one variation image 500 can be decomposed into its red, green, and blue (RGB) spectral components. This novel method has distinct advantages over previous cell counting techniques that were limited to grayscale images, since these decomposed image elements permit analysis of the image to be performed in the optimal spectral component so as to maximize the contrast of the target cells against the background, particularly when stains or



reagents are utilized. Use of multiple spectral components can also have the advantage of allowing multiple varieties of cells to be identified and distinguished in a single captured image. The spectral component that exhibits maximum contrast can then be selected for further processing by the image processor 106. In yet another embodiment, other target cell characteristics such as size or aspect ratio could be used to distinguish target cells from non-target cells.

**[0048]** Referring now to FIG. 7, shown therein is an exemplary flowchart of a method 700 of determining information concerning a target cell type in a sample, in accordance with aspects of the present disclosure. This information can be a cell count or a cell density. The method 700 may comprise a step 702 of providing a sample into an analysis chamber. The analysis chamber can have a stain or reagent material provided therein. The sample can be a hematology sample and may have a plurality of cell types, e.g., white blood cells, red blood cells, T-helper cells, etc., as well as the target cell type. The stain or reagent material may be selected so as to label the target cell types. For example, the stain or reagent may be adapted to render the target cell types more visible with respect to the other cell types in the sample. The target cell type may be a bacteria cell.

**[0049]** The method 700 may also include a step 704 of using cell-specific sorting/capture technique(s). This provides

isolation of desired cell types in a sample with cell plurality to enhance cell count accuracy. Step 704 may include antibody-specific reactions using surface chemistry or surface coating and using as size-exclusion channels.

**[0050]** The method 700 may also include a step 706 of shining a light through the analysis chamber. The light being projected may be from a large format light source. Step 708 includes capturing an image of the sample. The image may be created by an image sensor (e.g., the image sensor 306). The image sensor may be a charge coupled device sensor, another type of visual light sensor, an ultraviolet light sensor or an infrared sensor. Sources of wavelengths best suited to maximizing contrast for imaging a particular type of cell or bacteria should be used.

**[0051]** The method 700 may also include a step 710 of analyzing the image to detect a target cell type. The image may be analyzed by an image processor 106. The image can be analyzed to calculate the quantity or density of the target cell types labeled by the stain or reagent. The image may include information indicative of the diffracted shadow signal of the target cell type. The image may be processed by an image processor being implemented as a software module being executed on a computerized processing system. The method 700 further includes a step 712 of providing information concerning the target cell types in the sample. The

information may be output via a display device, a hard copy (e.g., print), a graph, chart, audibly, etc.

**[0052]** A card reader may be integrated with the digital processor for use with a card with a bar code or magnetic strip for the purpose of storing and/or retrieving information relating to the patient and sample, such as patient ID number, blood type, testing location, and cell counts. This unique feature of the invention would entail each patient tested to possess a card and insert it into the card reader slot at the beginning of the test process. The processor might retrieve information from a previous testing if existing, and store the results of the present test in a database associated with that card and thus the patient whom it belongs to.

**[0053]** As would be understood, utilizing the herein disclosed shadow imaging technique provides for automated counts such as lymphocyte, CD4<sup>+</sup>, or any other bacterial cell counts to be performed after whole blood has been injected into the micro-fluidic device 200, and/or 400. The image sensor 306 is placed under the micro-fluidic device wherein light emitted by the light source passes through a pinhole and reaches the surface of analysis chamber containing the sample. An image 500 captured by the image sensor 306 represents the diffracted shadow signal of cell shape.

**[0054]** This automated cell counting approach has certain advantages over traditional cell count techniques. It is applicable for cells under different illuminations, focal depths, and optical magnifications. The procedure is much faster (typically ~2-5 s on standard laptop) and more efficient than manual counting, which can require hours of labor. In addition, imaged cells can be detected and isolated where contrast is very low and/or background illumination varies substantially. Finally, the approach disclosed herein is relatively straightforward and requires minimal code to implement.

**[0055]** It will be understood that many additional changes in the details, materials, steps and arrangement of parts, which have been herein described and illustrated in order to explain the nature of the invention, may be made by those skilled in the art within the principle and scope of the invention as expressed in the appended claims.

**[0056]** The foregoing description of the preferred embodiments of the invention has been presented for purposes of illustration and description only. It is not intended to be exhaustive or to limit the invention to the precise form disclosed; and obviously many modifications and variations are possible in light of the above teaching. Such modifications and variations that may be apparent to a person skilled in the art are intended to be

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included within the scope of this invention as defined by the accompanying claims.

**IMAGING SYSTEM AND METHOD FOR BIOMEDICAL ANALYSIS**

**ABSTRACT OF THE DISCLOSURE**

An automated optical cytology system is provided that is suitable for analyzing samples in field conditions. The system (100) includes a micro-fluidic chip (102), an imager (104) and an image processor (106). The chip (200) includes an analysis chamber (218) for receiving a sample. The chip (200) may also include a compound provided in the analysis chamber (218) that can label a target cell type in the sample. The imager (104) includes a large format light source (308) which projects light through the analysis chamber (218) and an image sensor (306). The image sensor (306) provides an image to image processor (106). The image processor (106) analyzes the image to determine parameters related to target cell types in the sample.

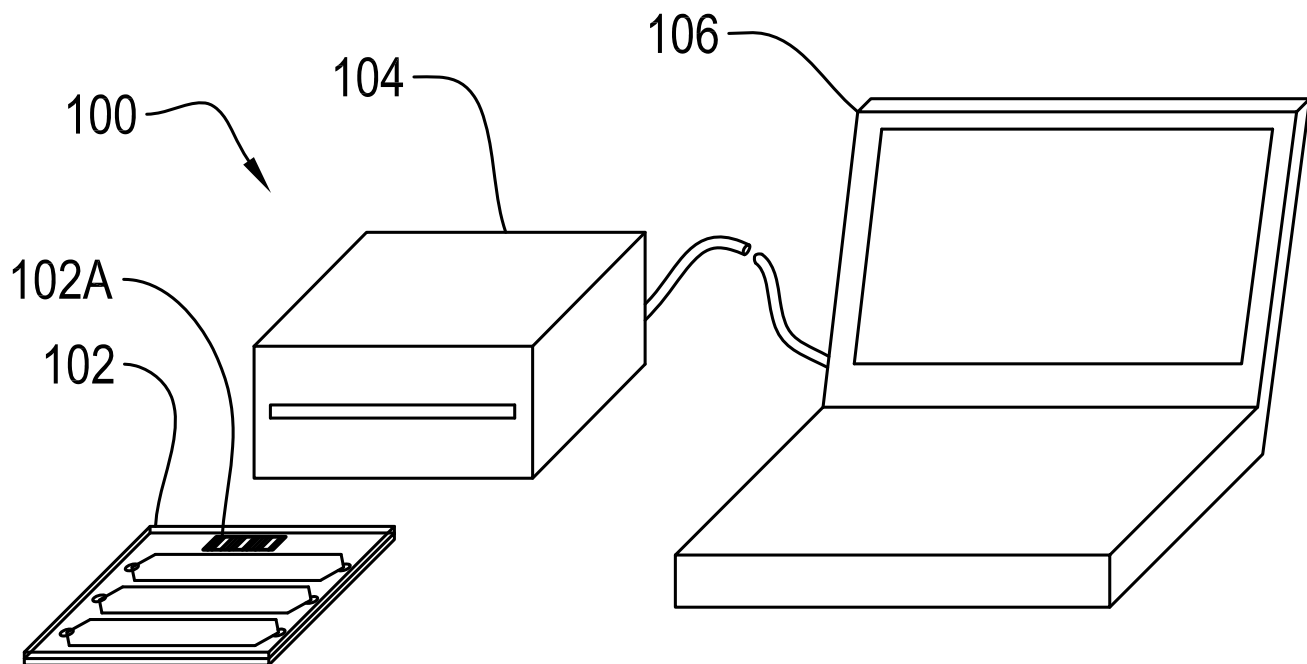


FIG. 1

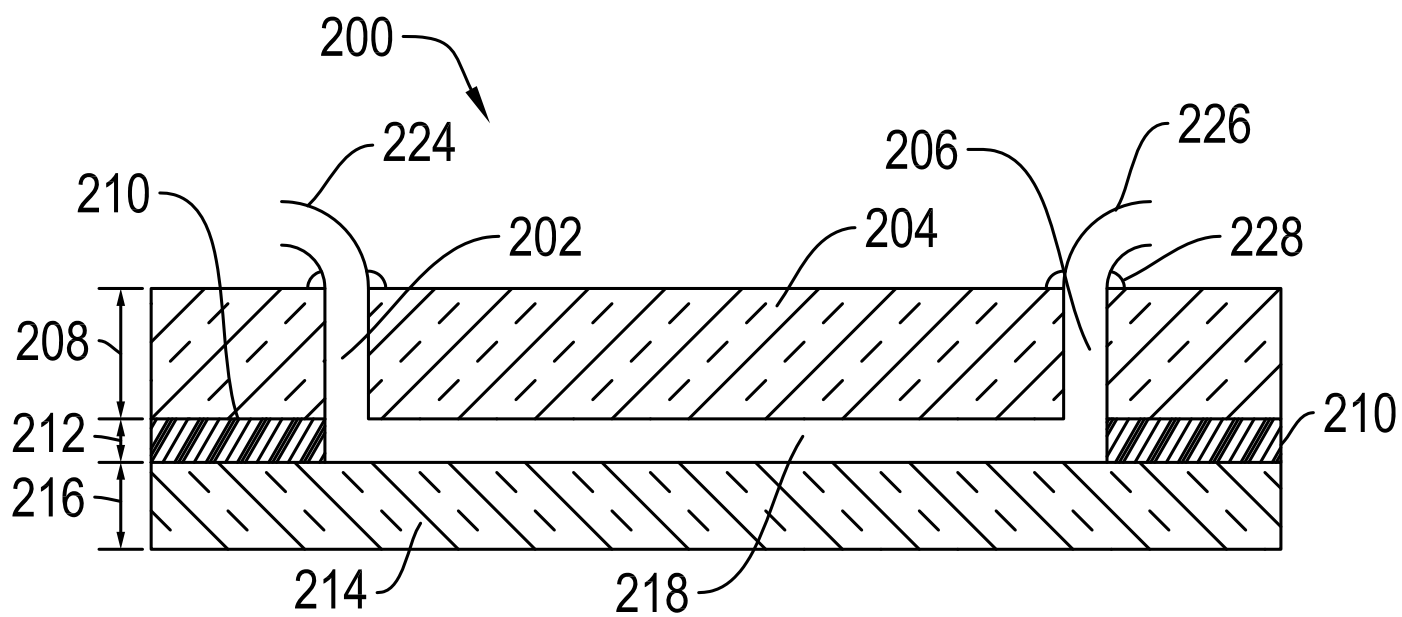


FIG. 2

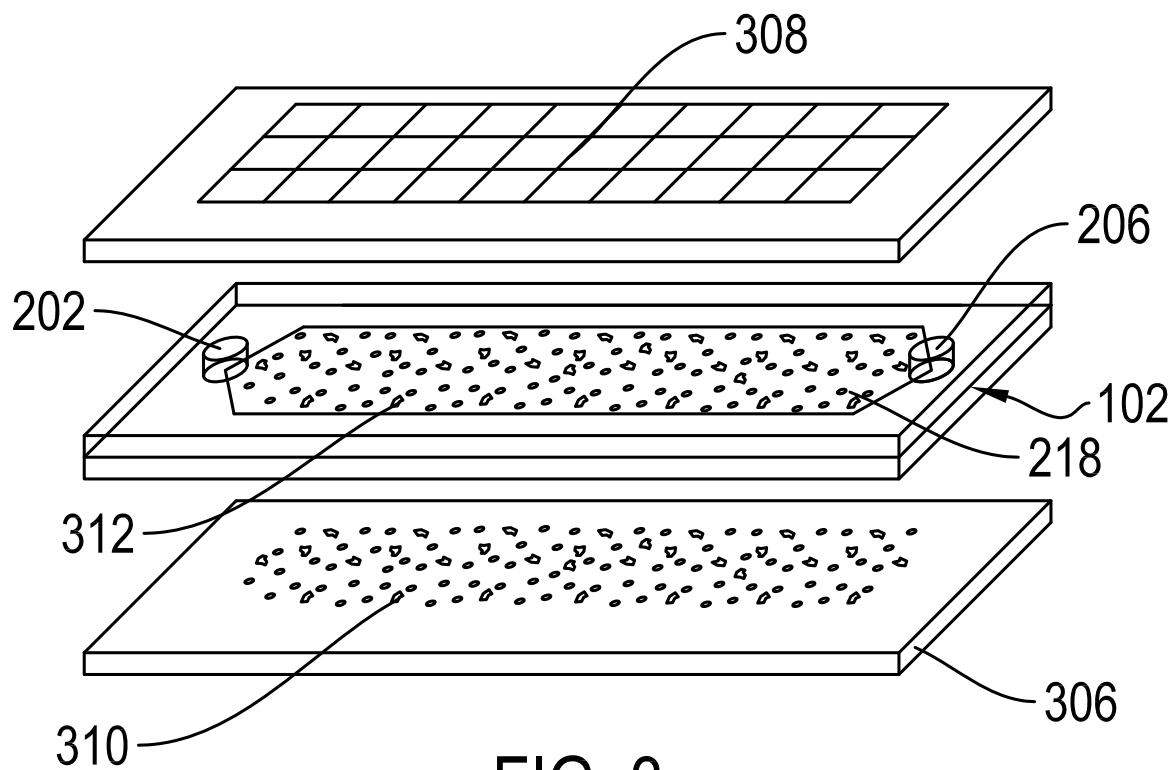


FIG. 3

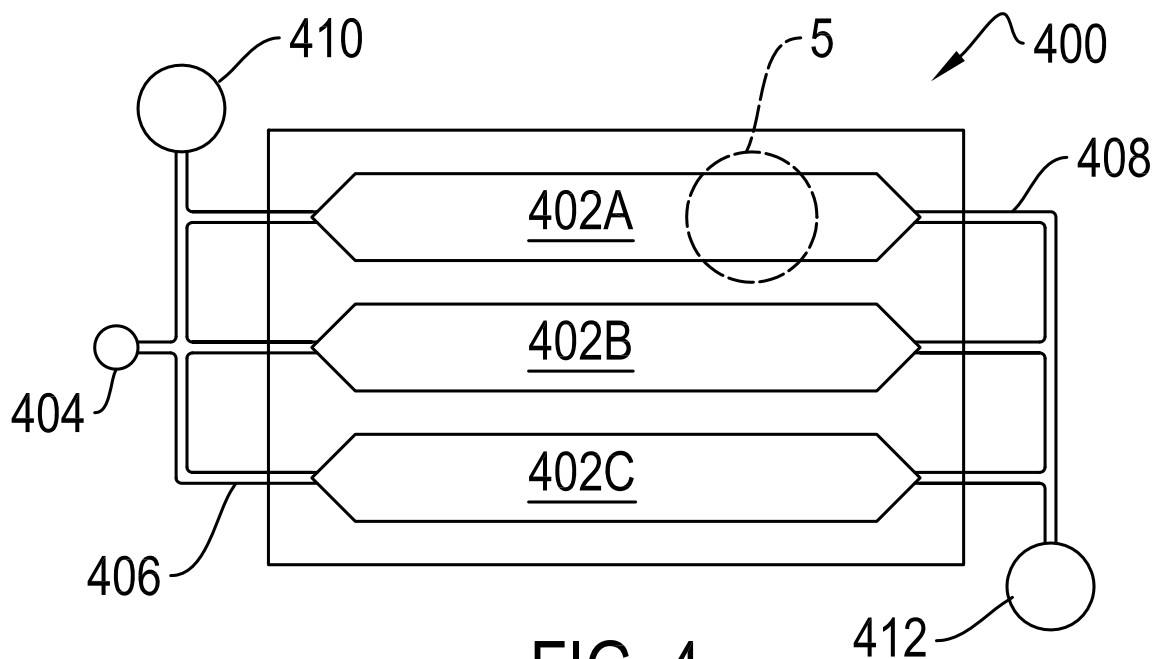


FIG. 4



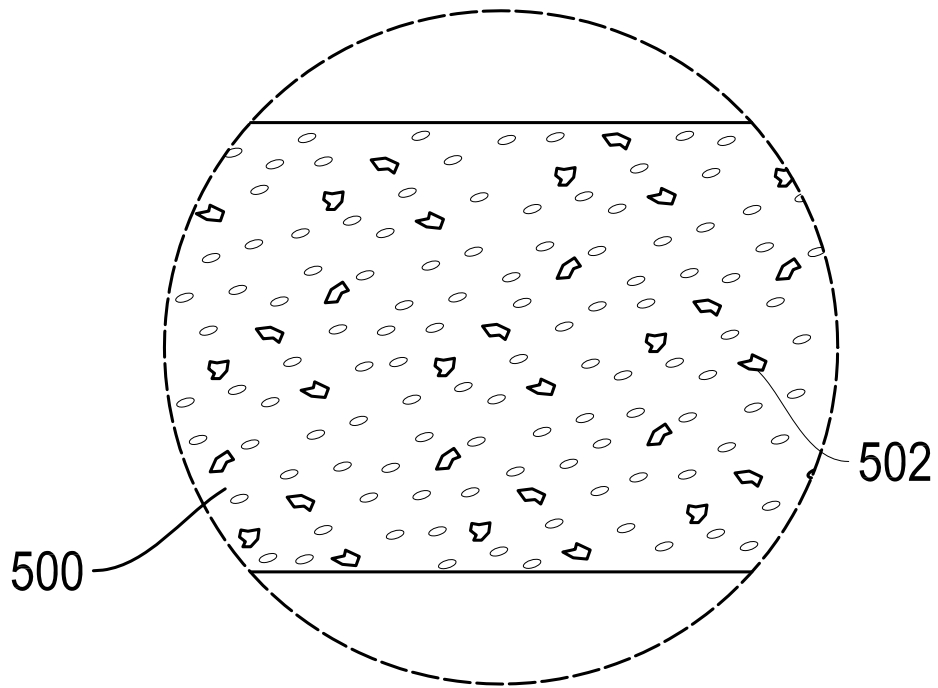


FIG. 5

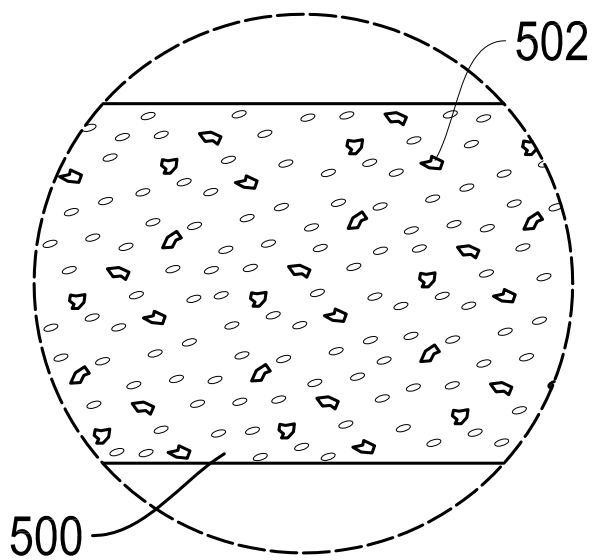


FIG. 6A

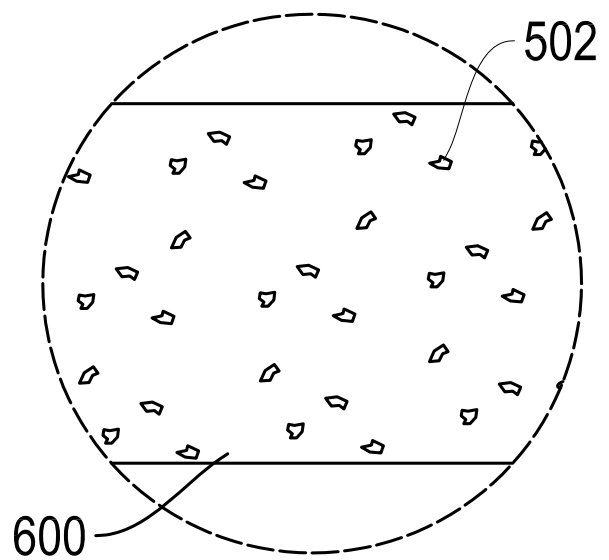


FIG. 6B

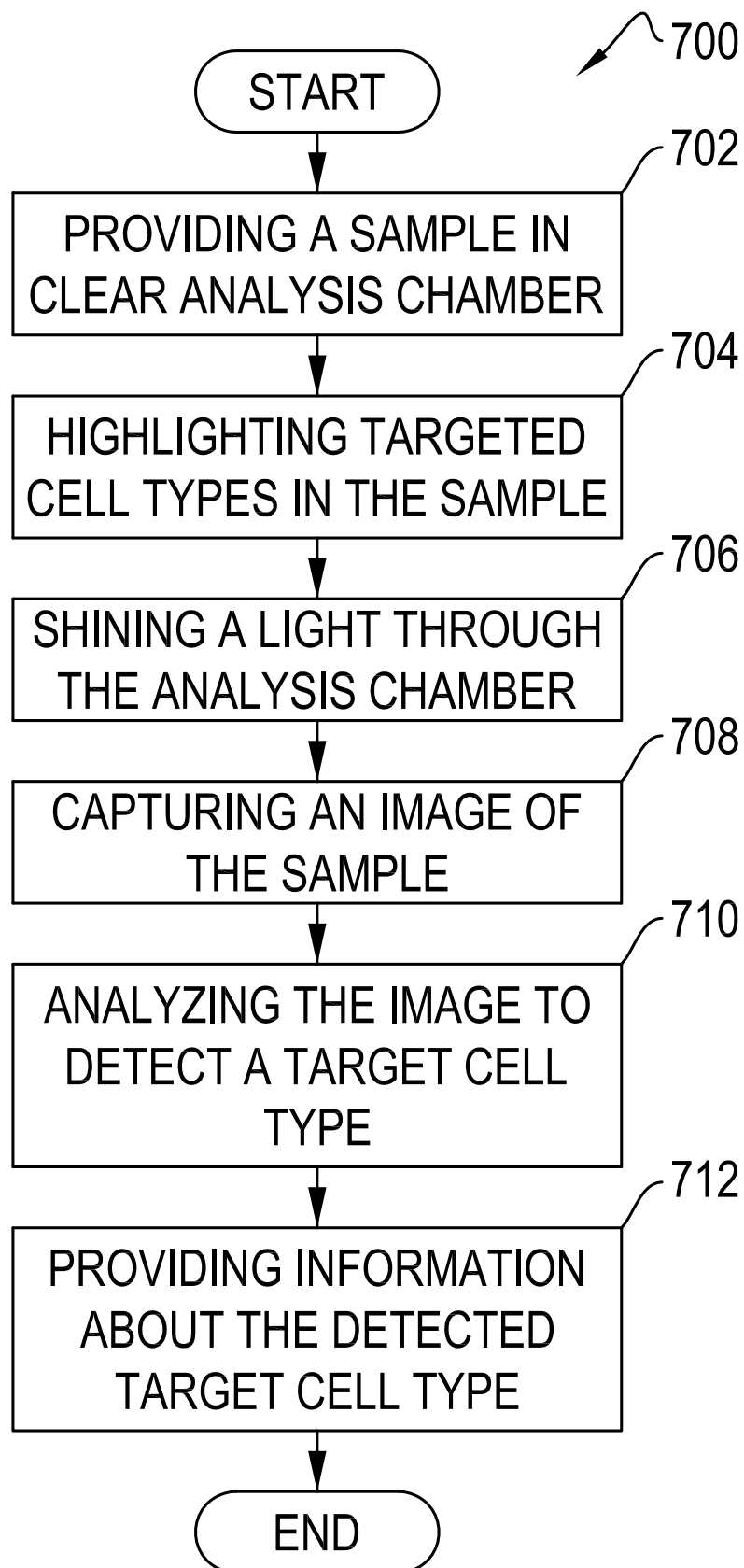


FIG. 7